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Development of bioanalytical parameters for standardization of *Terminalia arjuna*

Dinesh K. Patel[1,2], Kanika Patel[3], Dhanabal S.P.[2*]

[1]*Department of Pharmaceutics, Indian Institute of Technology, Banaras Hindu University, Varanasi-221005, India*
[2]*J.S.S. College of Pharmacy, Ooty- 643 001, India*
[3]*G.L.A Institute of Pharmaceutical Research, Mathura-281406, India*

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ABSTRACT

Objective: To develop a noval bioanalytical parameters for standardization of *Terminalia arjuna* (*T. arjuna*) extract. **Methods:** In the present investigation, parameters such as preliminary phytochemical analysis, total phenol, flavonoid, tannin content, solubility test, heavy metal analysis, antimicrobial study and quantitative analysis by HPTLC method were performed in order to standardization. **Results:** Preliminary phytochemical analysis showed the presence of alkaloid, carbohydrate, tannin, steroid, triterpenoid, glycoside, saponin, flavonoid, amino acid and protein. Loss on drying and solubily in water was found to be 5.04% and 81.10%. Total flavonoid and phenol content was found to be 10.4% and 0.44%. Total tannin content was found to be 24.8%. The content of quercetin and rutin in *T. arjuna* was found to be 1.08%w/w and 0.16%w/w respectively. Further the level of heavy metal and microrganism were found to be under the limit. **Conclusions:** These bioanalytical parameters can be used as an important tool for the food scientists, researchers and even the consumers for its standards.

1. Introduction

Phytochemicals are compounds mainly responsinle for different color, flavor and smell of plants and they play an important role in the plant's natural defense mechanism against various diseases[1]. Natural products offer unbounded opportunities for new drug development due to the chemical diversity. According to WHO, 80% of the World's population relies on traditional medicine to meet their daily health need. A large number of drugs prescribed worldwide are derived directly or indirectly from natural sources such as aspirin from *Filipendula ulmar* (*F. ulmar*), morphine from *Papaver sominiferum* (*P. sominiferum*) and ephedrin from ephedra. A number of plant based drug is included in the WHO's essential medicine list[2,3].

Terminalia arjuna (*T. arjuna*) belonging to family Combretaceae, was used in the Ayurvedic medicine since ancient times for the treatment of diseases. Plant parts, such as fruit and bark are mainly used to maintain good health[4]. *T. arjuna* bark powder and extract available as over-the-counter supplements for maintaining a healthy heart in the US. *T. arjuna* barks decrease blood pressure and heart rate, and counteract actions of norepinephrine and isoproterenol[5]. *T. arjuna* is appropriately known as "Hridya", as it possesses heart strengthening and cardiotonic properties. *T. arjuna* has been used in different forms such as Asava (alcoholic decoction), Ghrita (clarified butter), Kshirpka (boiled with milk) and dried bark powder for different medicinal purposes. It is also used to treat obesity, hypertension, ulcer and hyperglycemia. It has wound healing, antibacterial, antimutagenic/anticarcinogenic, antioxidant, hypocholesterolemic and antifertility activity[6-8]. *T. arjuna* is a famous Indian folk medicinal plant used as a cardiotonic in heart failure, ischaemic cardiomyopathy, atherosclerosis and myocardium necrosis. It is an essential ingredient of many Ayurvedic preparations which are sold as cardiotonics. It is used in the treatment of fractures, ulcers, blood diseases,

*Corresponding author: Dr. S. P. Dhanabal, Professor & Head, Department of Phytochemistry &. Phytomedicine, JSS college of Pharmacy, Ooty- 643 001, India.
Tel: 09443095697
E-mail: dhanskanaksahil@rediffmail.com

anaemia and asthma and has the ability to cure hepatic, congenital, venereal and viral diseases. Many oleanane type triterpenoids from *T. arjuna* plant showed antitumoral, antioxidant, antiallergic, antiasthmatic, antifeedant and cardioprotective activities[9,10].

Phytochemically *T. arjuna* contain tannin, saponin, ester, sugar, steroids, acids, triterpenoid saponins (e.g., arjunic acid and derivatives), ellagic acid, gallic acid, oligomeric proanthocyanidins, phytosterols, flavonoids (arjunone, arjunolone, luteolin), polyphenols, calcium, magnesium, zinc and copper[4,5,7]. Ursane triterpene glucosyl ester, 2 α , 3 β -dihydroxyurs-12, 18-dien-28-oic acid 28-O- β -D-glucopyranosyl ester, 2 α , 3 β , 23-trihydroxyurs-12,18-dien-28-oic acid 28-O- β -D-glucopyranosyl ester, quadranoside VIII, kajiichigoside F1 and 2 α , 3 β , 23-trihydroxyurs-12, 19-dien-28-oic acid 28-O- β -D-glucopyranosyl ester, as well as phenolic compounds, 3-O-methylellagic acid 4 ${}^{k}_{5}$ -O- α -L-rhamnopyranoside and (-)- epicatechinwere were isolated from *T. arjuna* plant[10,11].

2. Material and methods

2.1. Plant extract and Chemicals

Crude plant extract of *T. arjuna* was procured from Garlico Herbal Concentrate (M.P.), India. High Performance Thin Layer Chromatography (HPTLC) precoated plates Silica Gel Merck 60F_{254} was used as a stationary phase. Rutin and quercetin were used as a marker compound. All the chemicals and reagents used in the present analysis of analytical grade.

2.2. Development of bioanalytical parameters

A phytochemical screening was conducted on the *T. arjuna* extract using standard qualitative methods to conferm the presence of different phytoconstituents[12,13]. The presence of phytoconstituents in the extract was also analysed through TLC analysis[14]. Study of parameters such as solubility in water, tannin content, loss on drying, heavy metal analysis and microbiological assay were also performed as per method of IP, 1996 and WHO guidelines[15,16]. Total phenol and flavonoid content were also determined according to the standard methods[17,18]. Different combination of solvent system has been used for the optimization of solvent system for quantitative analysis through HPTLC methods.

2.2.1. Development of HPTLC methods for standardization

The quantification of rutin and quercetin in *T. arjuna* were determined through HPTLC. Different concentration of standard solution and extracts were applied on HPTLC plates. The HPTLC plates were developed in the optimized solvent system, dried in air and scanned at 254 nm using CAMAG TLC scanner 3. Further rutin and quercetin content was determined through advance high performance thin layer chromatography (HPTLC) method. For the preparation of the calibration curve in the quantitative analysis different concentration of the standard stock solution were prepared in the HPLC grade methanol. For the preparation of the samples solution, extract was dissolve in the methanol and then sonicated for 10 min and the final volume of the solutions was made up to 5 mL to get stock solutions. All the needed concentration of the samples was prepared from the stock solution by suitable dilution.

The chromatographic conditions for the HPTLC analysis used in the present investigation are as follows:

Analysis:	Estimation of rutin and quercetin in Terminalia arjuna extract.
Plate material:	HPTLC Precoated plates Silica Gel Merck 60F_{254}.
Solvent system:	Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26).
Syringe:	100 μL Hamilton (Bonadzu, Switzerland).
Application mode:	CAMAG Automatic TLC Sampler III.
Development mode:	Ascending.
Scanning:	CAMAG TLC scanner 3 with Cats software.
Experimental conditions:	Temperature (25±2) ℃, relative humidity 40%.

3. Results

Preliminary Phytochemical analysis showed that alkaloid, carbohydrate, tannin, steroid, triterpenoid, glycoside, saponin, flavonoid, amino acid and protein were found to be present. TLC analysis showed three spots R_f (0.28, 0.19, 0.06) in chloroform: methanol: ethanol (90:05:05) solvent system. Loss on drying and solubily in water was found to be 5.04%, 81.10%. The total flavonoid, phenol and tannin content were found to be 10.4%, 0.44% and 24.8%. Further heavy metal analysis was also performed in the current task and the level of lead, arsenic, mercury and cadmium were found to be under the limit. Microbiological assay was also performed in the current task and result showed that *E. coli* and salmonella was found to be absent whereas total bacterial count and yeast & moulds contents were found to be 445 and 56 CFU/GM.

Fingerprinting analysis of sample was done through HPTLC method, and the data were presented in the Table 1. For quantitative analysis through HPTLC techniques, optimization of solvent system was done using combination of solvent system of varying polarity and the most suitable solvent system were taken for the quantitative analysis. Ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26)

was found to be suitable solvent system for quantitative analysis through HPTLC. The content of quercetin and rutin in *T. arjuna* was found to be 1.08%w/w and 0.16%w/w respectively. The respective HPTLC chromatogram of rutin, quercetin, and *T. arjuna* extract were presented in the Figure 1, Figure 2 and Figure 3. The interpretations of result were done using standard calibration curve of quercetin and rutin (Figure 4 and Figure 5).The results suggest that the sample contained considerable amount of rutin, and quercetin.

Table 1

Fingerprint analysis of *T. arjuna* extract.

Solvent system	R_f value	Maximum peak height	Peak area (%)
n-butanol : acetic acid : H_2O (4:1:5)	0.14	248.8	24.40
	0.16	247.8	14.91
	0.28	15.3	0.76
	0.38	20.2	1.24
	0.56	66.3	7.06
	0.74	271.3	43.76
	0.86	41.1	3.23
	0.95	66.6	4.65

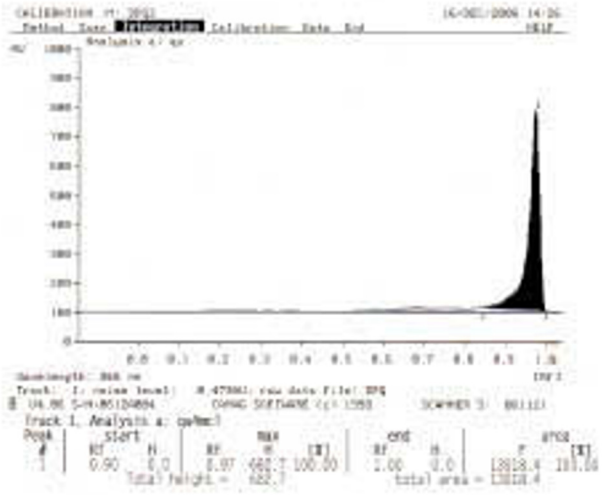

Figure 1. Standard HPTLC chromatogram of quercetin.

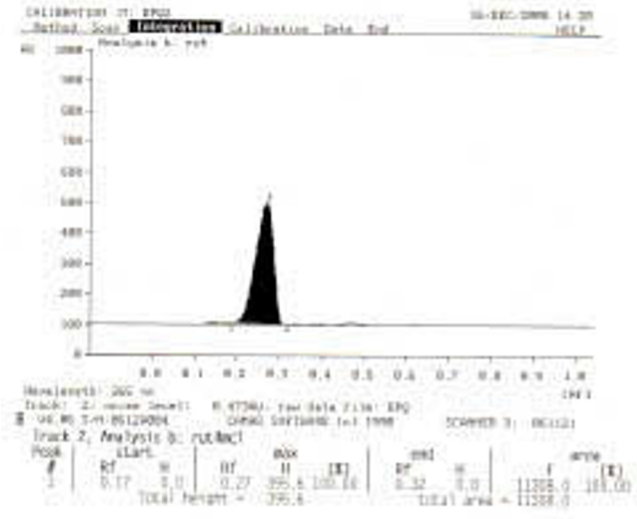

Figure 2. Standard HPTLC chromatogram of rutin.

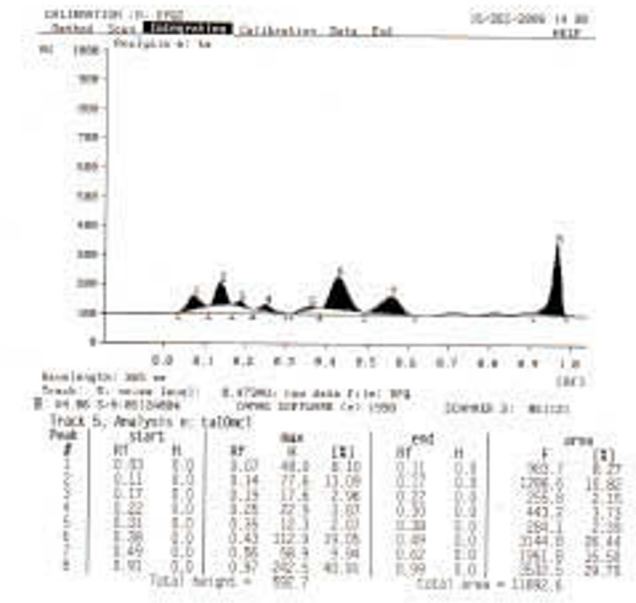

Figure 3. Standard HPTLC chromatogram of *T. arjuna* extract.

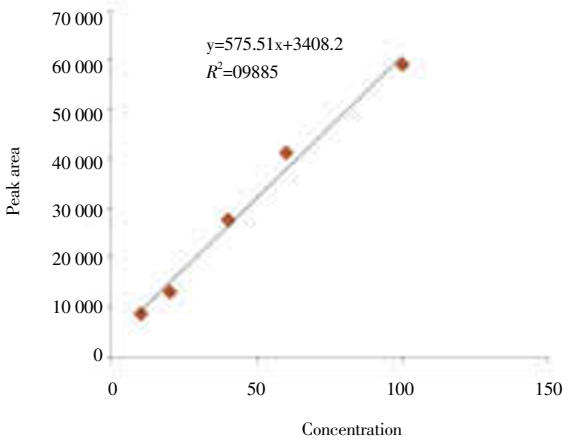

Figure 4. Standard calibration curve of quercetin.

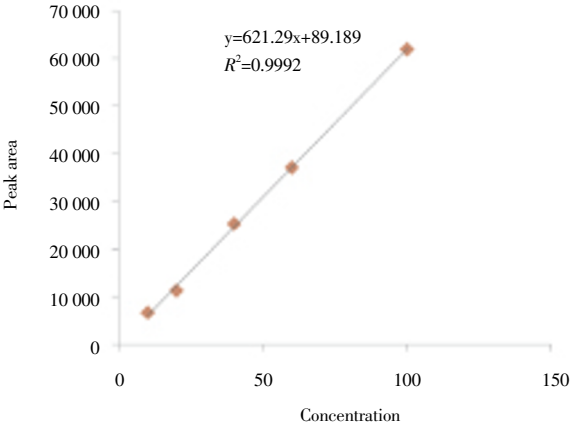

Figure 5. Standard calibration curve of rutin.

4. Discussion

Physicochemical and phytochemical analysis are used to check the genuine nature of the crude drug, thus it plays an important role in preventing the possible steps of adulteration[19]. Based on the reported traditional use in the literature, in the present investigation *T. arjuna* extract was taken.

The medicinal properties of plants material are mainly due to the presence of various phytoconstituents[20]. The presence of different phytoconstituents such as flavonoid, tannin, saponins, alkaloid and glycoside in the phytocohemical tests justifies their therapeutic potential[21-23].

These phytoconstituents have been reported to have multiple biological effects such as anti-inflammatory, anti allergic, antioxidant, antidiabetic, analgesic, antispasmodic, antibacterial, anti-viral, anti-cancer and aldose reductase inhibitory activities. It is also used for the treatment of diarrhea and dysentery[24-26]. Phytoconstituents obtained from natural sources have been gaining importance in the day by day due to the health promoting activity. So it is necessory to check the quality safety and efficacy of herbal drugs before its consumption[27,28].

Phytochemical standardization plays an important role to ensure the quality safety and efficacy of the herbal drug. In the last few decades, an HPTLC technique has gained much popularity for standardization of the herbal drugs and formulations. Analysis of several samples simultaneously using a small quantity of marker compound and mobile phase with very less time is the major advantage of HPTLC[28].

TLC and HPTLC techniques have been used as important analytical tools in pharmaceuticals, medicine, chemistry, food analysis, toxicology and environmental science[29]. Quality evaluation and standardization of the herbal preparation is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market[30].

These bioanalytical parameters can be utilizes for the simultaneous analysis of different phytoconstituents present in the *T. arjuna* plant material.

In future, this information may be useful as a standard to identify and to differentiate from its adultrants and other related species.

Conflict of interest statement

The authors report no conflict of interest.

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